

# IN THE CLAIMS:

Claims 1-52 (canceled).

Claim 53 (withdrawn) An isolated nucleic acid fragment encoding a putative protective antigen against Mycoplasma hyopneumoniae or related infections, said nucleic acid fragment including the following sequence, mutants, derivatives, recombinants and fragments thereof:

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
ATGAAAAAAA	TGCCACTATA	CCAGAGGAAA	GAGCAGTATA	TAAAATAATT	50
AAAATTACAT	TTTCTTCATT	TGCGCCAGAA	TTTTAAGAA	TTAGTACATT	100
AAAAAGTAGA	ACAAAAGTTA	TTAATGTAAA	CATTAGCGCA	ATCCTTAAGA	150
AAAAATTAAA	AGTTTATCT	ATTTTTTTA	ATCGAAATCC	AACCAGGCAT	200
AAATCTTTGT	CAGTATTTAT	CAAGTCGGTA	TTTTTTCATT	ATTTCTACTA	250
AAATATTATT	TGAATTTGCA	TTTTCCATAA	TCTAAAATTT	TACATTTTTT	300
TATAACAATT	TTTAAAAATT	ACTCTTTAAT	TTATAGTATT	TTTTTATTTT	350
TTAGTCTAAA	TTATAAAATT	ATCTTGAATT	TTATTTGAAT	TTTTATAATT	400
TAGTACTAAA	AAATACAAAT	ATTTTTTCCT	ATTCTAAGAA	AAATTCATTT	450
TTTAAAAAAA	ATTGATTTTT	ATAGTATAAT	TTGTTTGAT	AATTGAATTA	500
ACTTGATTTG	AAAGGGAACA	AAATGAAAAA	AATGCTTAGA	AAAAAATTCT	550
TGTATTCATC	AGCTATTTAT	GCAACTTCGC	TTGCATCAAT	TATTGCATTT	600
GTTGCAGCAG	GTTGTGGACA	GACAGAATCA	GGTTCAACTT	CTGATTCTAA	650
ACCACAAGCC	GAGACGCTAA	AACATAAAGT	AAGTAATGAT	TCTATTCGAA	700
TAGCACTAAC	CGATCCGGAT	AATCCTCGAT	GAATTAGTGC	CCAAAAAGAT	750
ATTATTTCTT	ATGTTGATGA	AACAGAGGCA	GCAACTTCAA	CAATTACAAA	800
AAACCAGGAT	GCACAAAATA	ACTGACTCAC	TCAGCAAGCT	AATTTAAGCC	850
CAGCGCCAAA	AGGATTTATT	ATTGCCCTGT	AAAATGGAAG	TGGAGTTGGA	900
ACTGCTGTTA	ATACAATTGC	TGATAAAGGA	ATTCCGATTG	TTGCCTATGA	950
TCGACTAATT	ACTGGATCTG	ATAAATATGA	TTGGTATGTT	TCTTTTGATA	1000
ATGAAAAAGT	TGGTGAATTA	CAAGGTCTTT	CACTTGCTGC	GGGTCTATTA	1050
GGAAAAGAAG	ATGGTGCTTT	TGATTCAATT	GATCAAATGA	ATGAATATCT	1100
AAAATCACAT	ATGCCCAAG	AGACAATTC	TTTTTATACA	ATCGCGGGTT	1150
CCCAAGATGA	TAATAATTCC	CAATATTTTT	ATAATGGTGC	AATGAAAGTA	1200
CTTAAAGAAT	TAATGAAAAA	TCGCAAAAT	AAAATAATTG	ATTTATCTCC	1250
TGAAGGCGAA	AATGCTGTTT	ATGTCCAGG	ATGAAATTAT	GGAAGTGGG	1300
GTCAAAGAAT	CCAATCTTTT	CTAACAATTA	ACAAAGATCC	AGCAGGTGGT	1350
AATAAAATCA	AAGCTGTTGG	TTCAAAACCA	GCTTCTATTT	TCAAAGGATT	1400
TCTTGCCCCA	AATGATGGAA	TGGCCGAACA	AGCAATCACC	AAATTAAAAC	1450
TTGAAGGGTT	TGATACCCAA	AAAATCTTTG	TAACTCGTCA	AGATTATAAT	1500
GATAAAGCCA	AAACTTTTAT	CAAAGACGGC	GATCAAATA	TGACAATTTA	1550
TAAACCTGAT	AAAGTTTTAG	GAAAAGTTGC	TGTTGAAGTT	CTTCGGGTTT	1600
TAATTGCAAA	GAAAAATAAA	GCATCTAGAT	CAGAAATCGA	AAACGAACATA	1650
AAAGCAAAAC	TACCAAATAT	TTCATTTAAA	TATGATAATC	AAACATATAA	1700
AGTACAAGGT	AAAAATATTA	ATACAATTTT	AGTAAGTCCA	GTAATTGTGA	1750
CAAAAGCTAA	TGTTGATAAT	CCTGATGCCT	AA		1782

Claim 54 (withdrawn) A method for producing an antibody against a Mycoplasma including providing a biological sample taken a short time after an immune animal has been challenged with a Mycoplasma or Mycoplasma extract taken from the infection site or an area of a lesion or an area close to the infection site or lesion:

isolating cells from the biological sample;

culturing cells in vitro in a suitable culture medium; and

harvesting antibodies produced from said cells.

Claim 55 (withdrawn) A method according to claim 54 wherein the biological sample is taken approximately 2 to 7 days after the animal has been challenged with the Mycoplasma.

Claim 56 (withdrawn) A method according to claim 55 wherein the culturing of cells in vitro further includes addition of helper factors to the culture, said helper factors selected from the group including cytokines used alone or in combination, including interleukin 1, 2, 3, 4, 5, 6, 7 and 8, colony stimulating factors, interferons and any other factors that may be shown to have an enhancing effect on specific B cell secretion.

Claim 57 (withdrawn) A method according to claim 56 further including a cell activation step including activating the cells isolated to proliferate and secrete and/or release antibodies, said cell activation step including adding a cell activating agent to the culture medium, said cell activating agent selected from the group including

mitogens and helper factors produced by leukocytes, or their synthetic equivalents or combinations thereof.

Claim 58 (withdrawn) A method according to claim 57 wherein the antibody is in the form of the supernatant harvested from the culture medium.

Claim 59 (withdrawn) An antibody against a Mycoplasma prepared according to the method of claim 54.

Claims 60- 66 (cancelled)

Claim 67 (withdrawn) A vaccine or veterinary composition including an antibody against a Mycoplasma according to claim 59.

Claims 68-69 (cancelled)

Claim 70 (withdrawn) An isolated DNA fragment encoding a putative protective antigen against Mycoplasma or related infections, said DNA fragment having a nucleic acid sequence according to Figure 6 or an homologous sequence, and functionally active fragments, mutants, variants or recombinants thereof.

Claim 71 (withdrawn) A clone including a DNA fragment according to claim 70.

Claim 72 (withdrawn) A clone according to claim 71 which is clone pC1-2.

Claims 73 - 74 (cancelled)

Claim 75 (currently amended) An isolated antigen prepared by a method comprising:

- (a) providing a sample of a *Mycoplasma*,
- (b) providing an antibody probe including at least one antibody against the *Mycoplasma*, said at least one antibody being produced by a method comprising
  - (i) providing a biological sample taken a short time after a mammal has been challenged with the *Mycoplasma* or an extract comprising the *Mycoplasma* at an infection or lesion site, said biological sample being taken from the infection or lesion site or an area close to the infection or lesion site;
  - (ii) isolating antibody producing cells from the biological sample;
  - (iii) culturing the isolated cells *in vitro* in suitable culture medium; and
  - (iv) harvesting the at least one antibody from said cultured cells;
- (c) probing the *Mycoplasma* sample with the antibody probe to detect at least one antigen; and
- (d) isolating the at least one antigen detected.

Claim 76 (previously presented) An isolated antigen comprising a molecular structure that is identifiable with an antibody probe produced by harvesting an antibody from antibody producing cells of a mammal that are at or close to an infection or lesion site within a short time after said mammal is challenged by infection with *Mycoplasma*

*hyopneumoniae* at said infection or lesion site, said molecular structure being a native *Mycoplasma hyopneumoniae* antigen having an approximate molecular weight in kilodaltons (kD) of between 110 - 114, 90 - 94, 72 - 75, 52 - 54 or 46 - 48, or being a mutant, derivative or fragment of the native antigen that stimulates production of the antibody in the antibody producing cells, wherein if the molecular structure is the native antigen having the molecular weight between 72 - 75 kD, the molecular structure contains an N-terminal amino acid sequence comprising SEQ ID NO:12, and wherein if the molecular structure has a molecular weight between 46 - 48 kD, the molecular structure has an N-terminal amino acid sequence comprising SEQ ID NO:3.

Claim 78 (previously presented) An isolated antigen according to claim 77, comprising at least one internal amino acid sequence selected from the group consisting of SEQ ID NO:13; SEQ ID NO:14 and SEQ ID NO:15.

Claim 79 (previously presented) An isolated antigen according to claim 76, wherein the molecular structure has a molecular weight between 60 - 64 kD and has an N-terminal amino acid sequence comprising SEQ ID NO:10 or SEQ ID NO:11.

Claim 80 (previously presented) An isolated antigen according to claim 76, wherein the molecular structure has a molecular weight between 52 - 54 kD and has an N-terminal amino acid sequence comprising SEQ ID NO:7.

Claim 81 (previously presented) An isolated antigen according to claim 80, comprising at least one internal amino acid sequence selected from the group

consisting of SEQ ID NO:8 and SEQ ID NO:9.

Claim 82 (previously presented) An isolated antigen according to claim 76, wherein the molecular structure has a molecular weight between 46 - 48 DK and has an N-terminal amino acid sequence comprising SEQ ID NO:3.

Claim 83 (previously presented) An isolated antigen according to claim 82, comprising at least one internal amino acid sequence from the group consisting of SEQ ID NO:4; SEQ ID NO:5 and SEQ ID NO:6.

Claim 84 (previously presented) A method of identifying an antigen associated with a *Mycoplasma*, said method comprising:

- (a) providing a sample of a *Mycoplasma*;
- (b) providing an antibody probe including at least one antibody against the *Mycoplasma*;
- (c) probing the sample with the antibody probe to detect at least one antigen; and
- (d) isolating the at least one antigen detected.

Claim 85 (previously presented) A method of purifying an antigen associated with a *Mycoplasma*, said method comprising:

- (a) providing a crude antigen mixture; and
- (b) providing an antibody against the *Mycoplasma* immobilized on a suitable support;

- (c) subjecting the crude antigen mixture to affinity chromatography utilizing the immobilized antibody; and
- (d) isolating the purified antigen so formed.

Claim 86 (currently amended) A method for preparing a synthetic antigenic polypeptide against *Mycoplasma*, which method comprises

- (a) providing a cDNA library or genomic library derived from a sample of the *Mycoplasma*;
- (b) providing an antibody probe produced by
  - (i) providing a biological sample taken a short time after a mammal has been challenged with the *Mycoplasma* or an extract comprising the *Mycoplasma* at an infection or lesion site, said biological sample being taken from the infection or lesion site or an area close to the infection or lesion site;
  - (ii) isolating antibody producing cells from the biological sample;
  - (iii) culturing the isolated cells *in vitro* in a suitable culture medium;
- and
- (iv) harvesting the at least one antibody from said isolated cells;
- (c) generating synthetic polypeptides from the cDNA library or genomic library;
- (d) probing the synthetic polypeptides with the antibody probe to detect the synthetic antigenic polypeptide; and
- (e) isolating the synthetic antigenic polypeptide detected thereby.

Claim 87 (previously presented) A method according to claim 86, wherein the at least one antibody is raised against an antigen from *Mycoplasma hyopneumoniae* or a related organism, said antigen being selected from the group of native *Mycoplasma* antigens having approximate molecular weights of 110 - 114, 90 - 94, 72 - 75, 52 - 54 and 46 - 48 kilodaltons (kD) or being a mutant, derivative or fragment of a native *Mycoplasma* antigen that stimulates production of the at least one antibody in said mammal.

Claim 88 (previously presented) A synthetic antigen produced by the method of claim 86.

Claim 89 (previously presented) A vaccine or veterinary composition comprising a prophylactically effective amount of at least one antigen according to claim 76.

Claim 90 (previously presented) A vaccine or veterinary composition comprising prophylactically effective amounts of a plurality of antigens according to claim 76.

Claim 91 (previously presented) A diagnostic kit including an antigen according to claim 76.

Claim 92 (previously presented) A method for preventing or treating *Mycoplasma* infection, which method comprises administering to a mammal a prophylactically or therapeutically effective amount of at least one antigen according to claim 76.



Claim 93 (currently amended) An amino acid sequence encoded by ~~a DNA fragment~~ ~~comprising~~ SEQ ID NO:1 ~~or a homolog thereof~~ or a functional equivalent of said amino acid sequence.

Claim 94 (currently amended) An amino acid sequence ~~comprising~~ consisting of SEQ ID NO:2 or a functional equivalent of said amino acid sequence.

Claim 95 (new) The isolated antigen according to claim 75, wherein the biological sample provided in (b)(i) is taken from the mammal within about 2 to 5 days after the mammal has been challenged with the *Mycoplasma* or extract at the infection or lesion site.

Claim 96 (new). The isolated antigen according to claim 76, wherein the antigen is identifiable with the antibody probe produced by harvesting the antibody from the antibody producing cells of the mammal within 2 to 5 days after the mammal is challenged with the *Mycoplasma hyopneumoniae* at the infection or lesion site.

Claim 97 (new). The method according to claim 86, wherein the biological sample provided in step (b)(i) is taken from the mammal within about 2 to 5 days after the mammal has been challenged with the *Mycoplasma* or extract at the infection or lesion site.

Claim 98 (new). The amino acid sequence according to claim 93, wherein the amino acid sequence is encoded by SEQ ID NO: 1.